A QUANTITATIVE STUDY OF NUCLEIC ACIDS IN THE LIVER TISSUE OF A/JAX MICE

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by

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CHAPTER I

INTRODUCTION

Previous studies have indicated that carbon tetrachloride administered orally or intraperitoneally causes cirrhosis of the liver and hepatomas. In an attempt to explain the development of cirrhosis and hepatomas some investigators have made quantitative analyses of the nucleic acid present in the liver tissue of mice after a single feeding of carbon tetrachloride[1] and some after a series of feedings.[2] Most of these investigations indicate some very definite changes in the amount of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

The fact that these changes occurred may indicate the effects of carbon tetrachloride on the liver cells, or may be evidence that these substances vary to marked degree from one organism to another even if they were not subjected to the effects of carbon tetrachloride.

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[1] R. E. Stowell, and C. S. Lee, "Histochemical Studies of Mouse Liver After Single Feedings of Carbon Tetrachloride," AMA Arch Path L (1950), 519-537; and K. K. Tsuboi, R. E. Stowell, and C. S. Lee, "Chemical Alterations Induced in Mouse Liver Following a Single Feeding of Carbon Tetrachloride," Cancer Res. II (1951), 87-93.

[2] D. S. Dudley, W. H. Coppock, and L. P. Johnson, "RNA, DNA, Lipid Phosphorous, and Acid Soluble Phosphorous in Normal A/Jax Mouse Livers," Proc. Iowa Acad. Sci. LXVI (1959), 426-431.

It has been established that DNA is the gene substance responsible for inherited characteristics, and there is common agreement that RNA is important in passing instructions from the nucleus to the cytoplasm of a cell. It has also been established that these characteristics do not all become active at the same time, but seem to be activated differently in response to sex, age, enzymes, and the release of hormones during various stages of development.

The present investigation was undertaken in an attempt to establish if some relationship in the DNA content of untreated mice of both sexes existed at various ages. Such information is an essential base for comparing the DNA and RNA content of carbon tetrachloride treated and untreated mice.

CHAPTER II

HISTORY

Early investigations of the effect of carbon tetrachloride on the liver tissue of mice indicate that the concentration of the nucleic acids DNA and RNA fluctuate (Figure I). The DNA content increased sharply after one feeding through the sixth feeding then decreased to a medium level following an increased number of carbon tetrachloride feedings.[1] This increase was not consistant. Tsuboi, Stowell and Lee[2] indicate the results of a "one-feeding study" shows an initial decrease followed by a sharp rise.

A study of the results of bi-weekly feedings of carbon tetrachloride[3] also indicated a decrease in DNA content following the initial feeding. However, this is reversed with continued feeding of carbon tetrachloride. The reversal does not indicate consistency, but varies at

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[1] L. J. Carlson, D. J. Johns, R. F. Morrison, L. D. Spriggs, and E. F. Suters, "DNA, RNA, Lipid Phosphorous and Acid Soluble Phosphorous in Livers of A/Jax Mice Fed Carbon Tetrachloride", (1960) Unpublished Drake University Biology Dept. (typed manuscript).

[2] Tsuboi, op. cit., p. 89.

[3] R. E. Stowell, C. S. Lee, K. K. Tsuboi, and A. Villasana, "Histochemical and Microchemical changes in Experimental Chirrhosis and Hepatoma Formation in Mice by Carbon Tetrachloride", Cancer Res. XI (1951), pp. 345-354.

intervals until it reaches its maximum, with a final average of 316 mg per 100g of tissue.

Laquerrier's[1] investigation of mice after partial hepatectomy gives an insight into the changes associated with damage to the liver tissue of mice. He states that "removal of forty per cent of the liver tissue produced a measurable increase in the DNA content of the remaining portion of the liver and this increase was a prelude to the initiation of the prophase of mitosis."

In contrast to the previous reports, Borning, Richter and Funder[2] reported results of an analysis of nucleic acids two hours after a single injection of carbon tetrachloride which indicates no appreciable change either in the number of nuclei or the DNA content in the liver cells of mice. The reports do indicate, however, a slight increase in the DNA content.

The RNA content of the cells have been reported to

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1 R. Laquerriere, "Changes in Deoxyribonuleic Acid in the Livers of Mice After Partial Hepatectomy", Pathol. et biol., Semaine Hop. VII (1959), pp. 1939-41.

2 H. Bornig, G. Richter, and H. Funder, "Metabolism of Damaged Tissues. XII Mitochondria and Nucleic Acids in the Cell Fractions of the Carbon Tetrachloride Damaged Liver of Mice," Z. Physol. Chem. CCCXXII (1960), pp. 213-230, cited in CA. LV (June 26, 1961), 12612d.

vary to a much greater degree than does the DNA. Lamirande, Allard and Cantero[1] investigated the RHA content of the cytoplasm in the livers of nine rats, and report a "continuous variation from one fraction to another.

Other investigations of RNA have reported the fluctuations in quantity of materialy begin with an initial decrease. However, following this initial decrease the nucleic acid shows a rapid recovery that surpasses the initial starting point, but this recovery is followed by another decrease. One such study carried on at Drake University[2] used A/Jax mice of both sexes. These mice were fed, by means of a capillary dropping pipette, o.1 ml of forty per cent v/v carbon tetrachloride in olive oil three times weekly for various lengths of time.

The mice were then analyzed to determine the concentration of the nucleic acid fractions. The results of the analysis show the untreated mice used as control has 730 mg of RNA per 100g of tissue. The mice subjected to one feeding illustrated a lower concentration of RNA, 675 mg per 100g of tissue.

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[1] G. de Lamirande, C. Allard, and A. Cantero, "Ribonucleic Acid Composition in Cytoplasmic Fractions Isolated From Rat Liver Cells," J. Biophys. Biochem. Cytol. VI (1959), pp. 291-292.

[2] Carlson, op. cit., Manuscript.

After this decrease the quantity of RNA, increased after six feedings to 775 mg per 100g of tissue. The RNA exhibited its greatest decrease at eleven feedings (400 mg/100g of tissue); after eighteen feedings the RNA recovered to almost the same value recorded for the untreated mice (770 mg/100g of tissue). The RNA concentration (580 mg/100g of tussue) observed after twenty-two feedings indicates the level of RNA when tumors became macroscopic. The rapid increase from this point levels off after thirty-six feedings at 793 mg/100g of tissue and remains at 703 mg through the forty-second feeding. From the 703 mg, the RNA concentration decreased to a final reading of 622 mg for fifty-four feedings. The average concentration of RNA for all organisms tested was 675 mg per 100g of tissue.

The concentration of DNA varied less than did the RNA. The lowest concentration recorded for DNA was observed in the untreated mice. From this low of 323 mg/100g of tissue the maximum concentration of 549 mg occurred after the sixth feeding of carbon tetrachloride. There are other peaks of DNA concentration (twenty-nine feedings --481 mg; forty-two feedings--486 mg). None of these approach the high value observed after the sixth feeding.

The feedings of carbon tetrachloride which caused the lowest concentrations of DNA in the liver tissue are: eighteen (390 mg), thirty-six (402 mg), and forty-eight (421 mg). These are all below the calculated average of

431 mg/100g of tissue (Figure I).

The mice used in the previous reports ranged in age from four weeks[1] to twenty-four weeks[2] and, with the exception of one investigation,[3] the mice studies were all males. The strain of mice used in each investigation varied and the data of the different strains are summarized in Table I.

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[1] C. P. Barnum, C. W. Nash, E. Jennings, O. Nygaard, and H. Vernual "The Separation of Pentose and Desoxypentose Nucleic Acids From Isolated Mouse Liver Cell Nuclei," Arch. Biochem. XXV (1950), pp. 376-383.

[2] A. J. Baxi, K. D. Samarth, and P. R. Venkatarman, "Effects of a Single Injection of Thyroxine of the Protein and Nucleic Acids of the Mouse Liver and the Protein of the Mouse Carcass", Proc. Indian Acad. Sci. XXXIV (1952), pp. 258-266.

[3] Dudley, op. cit., pp. 426-431.

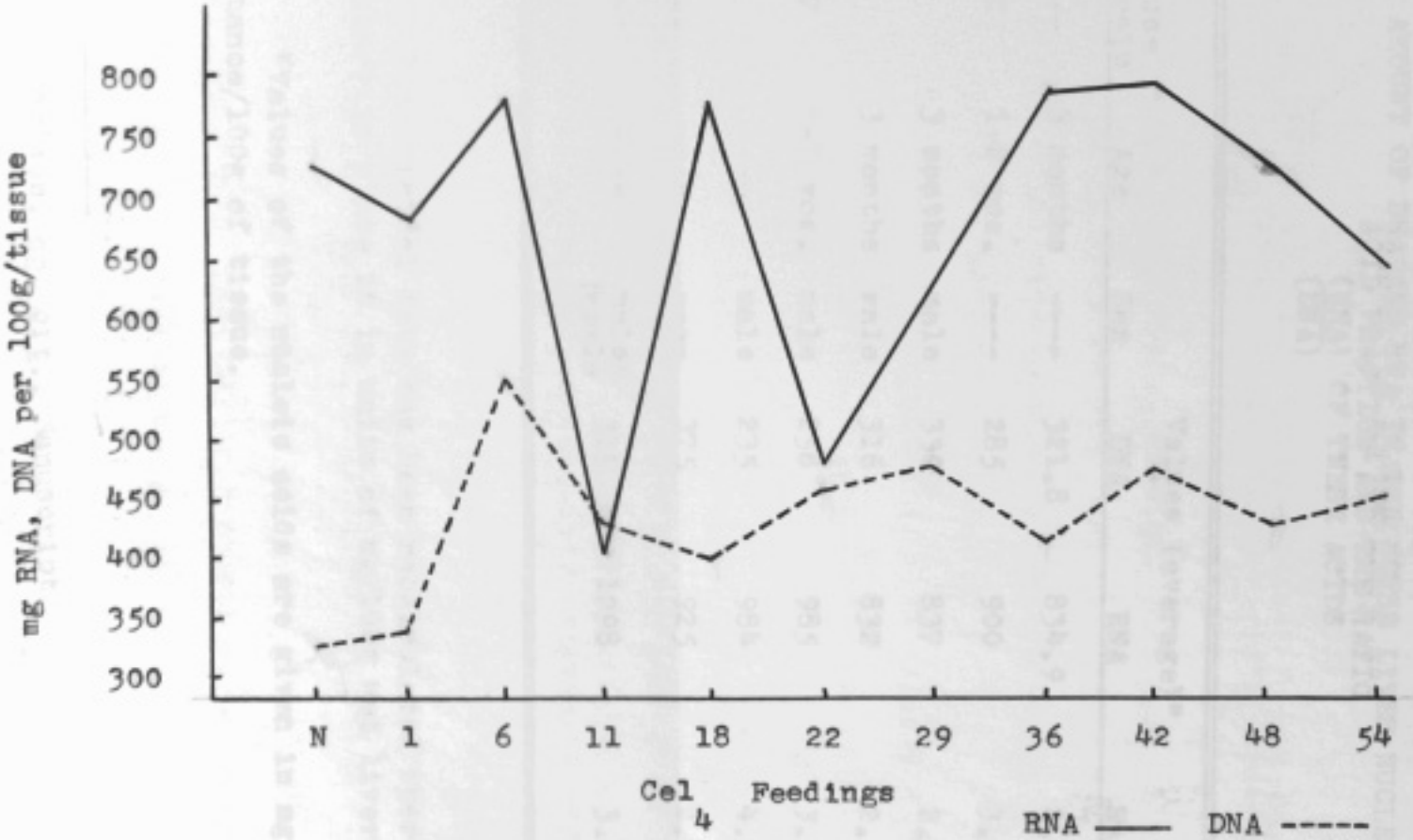

Figure 1. RNA, DNA concentration in mice fed carbon tetrachloride.

TABLE I

AMOUNT OF DNA AND RNA IN THE MOUSE LIVER NUCLEIC ACID FRACTION[1] AND THE RATIO $\left(\frac{RNA}{DNA}\right)$ OF THESE ACIDS

Mouse Strain	Age	Sex	Values (average)* DNA	RNA	Ratio
----	3 months	----	321.8	834.9	2.64
ZBC	1-2 mos.	----	285	900	3.16
A	3 months	male	330	837	2.53
A	3 months	male	316	832	2.63
C-57	6-7 mos.	male	258	985	3.81
dba	----	male	235	984	4.18
white	----	----	375	925	2.46
A/Jax	----	male& female	319	1098	3.45

The original data has been recalculated where necessary to place it in units of mg/100g wet liver tissue.

*Values of the nucleic acids are given in mg of substance/100g of tissue.

[1] Carlson, op. cit., Manuscript.

CHAPTER III

MATERIALS AND METHODS

A/Jax mice of both sexes obtained from Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine, were used throughout the experiment. Previous work done at Drake University using the same strain of mice illustrated the tendency of these mice to develop hepatic tumors if carbon tetrachloride was administered.

This research was initiated with A/Jax mice which were untreated with carbon tetrachloride, but were otherwise maintained the same as the mice used in the previous investigations. Males and females of various ages were used, and compared in an attempt to consider as many variables as possible for correlation between this work and investigations carried on in the past.

The mice were weighed and killed by cervical dislocation. The liver was removed immediately and weighed on a Roller-Smith balance. Sufficient distilled water was added to make a twenty per cent homogenate and the mixture was ground in a teflon homogenizer to uniform consistency. Three 1 ml aliquots of the uniform homogenate were transferred to three-inch test tubes. The homogenizer and test tubes were kept in an ice bath until extractions

were started. The procedure followed was a slight modification of Schneider.[1]

To the 1 ml aliquots of cold homogenate, 2.5 ml of cold 10 per cent TCA (trichloroacetic acid) were added, and the samples were shaken vigorously for five minutes. They were then centrifuged and the supernatant removed by bulb pipettes and discarded. Extraction was repeated in the same manner and again discarded. This completed the extraction of the acid soluble phosphates.

The residue from above was extracted with 5 ml of 1:4 water:95 per cent ethanol; the mixtures were shaken and centrifuged, and the supernatant pipetted and discarded. A second extraction with 5 ml of 95 per cent ethanol was similarly treated to conclude the extraction of the phospholipids.

The two phosphate extractions were discarded because this investigation did not concern itself with the phosphates found in the liver, but only with the nucleic acid fractions, Deoxyribonucleic acid and Ribonucleic acid. However, since the original research included an analysis of the phosphates, this investigation included the phosphate extraction in an attempt to duplicate the procedures used in the original research.

To the residue from the phosphate extractions 2.5 ml

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[1] W. C. Schneider. "Phosphorous Compounds in Animal Tissue and Extraction and Estimation of Desoxypentose Nucleic Acid and of Pentose Nucleic Acid", J. Biol. Chem. CLXI (1945), pp. 293-302.

of cold 5 per cent TCA was added and the mixture was shaken and centrifuged, and the supernatant pipetted off into a 10 ml volumetric flask for each aliquot. A second extraction with 5 ml of 5 per cent TCA was heated at 90o C. for twenty minutes, cooled in running water, centrifuged, and the supernatants removed to respective flasks and combined with that of the first extraction. A third extraction with 2.0 ml of 5 per cent TCA (room temperature) was centrifuged after five minutes of shaking, and supernatant combined in the appropriate flasks with those from the previous extractions. The respective flasks were then diluted to the mark with 5 per cent TCA (room temperature).

Three ml of each of the three aliquots of the RNA and DNA extract were placed in six-inch test tubes. The tubes were labelled D_1, D_2, and D_3. Three ml of 5 per cent TCA to be used as a blank and 3 ml of a standard DNA solution (12 mg/100 ml) were placed in two other six-inch test tubes. Six ml of diphenylamine indicator solution was added to each test tube and they were heated in a boiling water bath for three minutes and allowed to stand overnight in the dark. The next morning they were read on the Coleman Spectophotometer for optical density at 600 mm using square cuvettes and using the blank to zero the machine and comparing each sample with the standard. The results were recorded and the DNA in mg/100g of tissue were calculated as follows:

$$\text{mg DNA} = \frac{\text{O.D. sample}}{\text{O.D. standard}} \times \frac{\text{12 mg DNA}}{\text{100 ml of standard}} \times \frac{\text{10 ml of solution}}{\text{aliquot}} \times \frac{\text{1 aliquot}}{\text{.197g of tissue}} \times \frac{\text{100g tissue}}{\text{100 g tissue}}$$

Three ml of each three aliquots of the combined DNA, RNA extract were placed in three six-inch test tubes. The test tubes were labelled R_1, R_2, and R_3. Into each of these tubes, 3 ml of 5 per cent TCA was added to dilute each sample. In similar tubes, 6 ml of 5 per cent TCA was used as a blank, and 3 ml of standard RNA solution (16 mg RNA/100 ml) with each 3 ml of 5 per cent TCA as a dilutent used as a standard. Into each of the five test tubes was placed 6 ml of orcinol indicator solution, and the tubes were heated in a boiling water bath for twenty minutes, cooled in running water, and read on the Coleman set at 620 mm after the machine was set at zero with the blank and each reading compared to the standard. The results were recorded and the mg RNA/100g of tissue were calculated as follows:

$$\frac{\text{mg RNA}}{\text{100 g of tissue}} = \frac{\text{D sample} - .00405\ (\text{mg DNA/100 ml})}{\text{D standard}} \times \frac{16}{100} \times$$

$$\frac{10}{1} \times \frac{100}{.197} \times \frac{\text{mg DNA}}{\text{100 ml}} = \frac{\text{12 x density of DNA sample}}{\text{density of DNA standard}}$$

The .197g instead of .2g calculated from the 1 ml of 20 per cent homogenate was based on the actual weight of

tissue delivered from the 1 ml serological pipettes used to make the aliquots by taking twenty per cent of the weight actually delivered.

The reagents and solutions used in the analysis were prepared as follows:

1. 10%w/v TCA - 100g of trichloroacetic acid dissolved in enough distilled water to make one liter of solution.
2. 5% w/v TCA - 10%w/v TCA mixed with an equal volume of distilled water.
3. 1:4 (H_2O - 95% v/v ethanol) 800 ml of 95% v/v ethanol added to 200 ml of distilled water.
4. 95% v/v ethanol - commercial available form.
5. Standard DNA solution - $\frac{12 \text{ mg DNA}}{100 \text{ ml solution}}$: 50 mg of DNA from General Biochemicals, Inc., Lot No 8044c; dissolved in three ml of one normal KOH in a preweighed thirty ml beaker by adding the solution to the dry DNA weighed in the beaker. Transfer quantitatively to a twenty-five ml volumetric flask and dilute to mark with distilled water. Six ml of this concentrate is transferred by pipette to a 100 ml volumetric flask. An equal volume (6 ml) of 10 per cent is added and the flask is filled two-thirds full with 5 per cent TCA. The flask is heated in a water bath at 90°

C. for fifteen minutes, and allowed to cool until the next day and then diluted to volume with 5 per cent TCA.

6. DNA Indicator Solution: 2.5g of diphenylamine (1% v/v) is added to sufficient glacial acetic acid to prepare 250 ml of the solution to which 6.9 ml of concentrated H_2SO_4 is added.

7. RNA standard Solution - $\frac{16 \text{ mg}}{100 \text{ ml of solution}}$: 50 mg of RNA from General Biochemicals, Inc., Lot No. 8045c is weighed in a previously weighed thirty ml beaker. Three ml of 1 N KOH is added to dissolve the RNA, which is then transferred quantitatively to a 25 ml volumetric flask and diluted to the mark with distilled water. Eight ml of this concentrate is transferred by pipette to a 100 ml volumetric flask. Eight ml of 10 per cent TCA is added, and then the flask is filled two-thirds full with 5 per cent TCA and heated to 90° C. for fifteen minutes, and allowed to stand until the next day before diluting to volume with 5 per cent TCA.

8. RNA Indicator Solution: dissolve 0.500g of orcinol (1,3-dihydroxy-5methylbenzene) in a little less than 250 ml of 0.00500 per cent $CuCl_2$ in concentrated HCL (50.0 mg of $CuCl_2$ per liter of

solution with concentrated HCL as the solvent) and dilute to mark in a 250 ml volumetric flask.

CHAPTER IV

RESULTS AND INTERPRETATION OF DATA

Forty mice were studied for the determination of the concentration of DNA and RNA. Each week the livers of two untreated A/Jax mice were removed and homogenated after the mice has been sacrificed by cervical dislocation. Forty mice were studied, but only thirty-four were used as a source of data. The other six mice were sacrificed for the purpose of reperfecting the technique of extraction.

The color of the liver was compared with the livers of the mice treated with carbon tetrachloride in a previous study. In this investigation the livers appeared to be a bright red rather than the gray-red observed in the mice fed carbon tetrachloride. The livers of mice in the present study were easily homogenized in contrast to the difficulty encountered with homogenizing livers of mice treated with carbon tetrachloride. Less connective tissue is present in livers of untreated mice.

In the chemical analysis, quantities of both nucleic acids, DNA and RNA, fluctuated (Figure II). The RNA concentration possessed the greatest variance. The average value for the mice five weeks of age was 666 mg per 100g of tissue. At six weeks of age the RNA concentration decreased to 657 mg, and from this reading the RNA dropped to

its lowest concentration, 493 mg per 100g of tissue at seven weeks of age. The increase in the concentrations of RNA is abrupt from this point, age seven weeks. Concentrations of RNA are: 635 mg at eight weeks, 647 mg for nine weeks, and 738 mg at ten weeks. A slight decrease was noted at eleven weeks of age. The RNA content fell to 718 mg per 100g of tissue; however after this slight decrease the RNA concentration increased to its highest value, at twelve weeks of age the RNA content is 854 mg per 100g of tissue. The decrease to 660 mg at thirteen weeks of age is followed by an increase to 791 mg at fourteen weeks, where the concentration is relatively constant for the next two readings, 791 mg for fifteen weeks and 797 mg at sixteen weeks of age. Another decrease of RNA concentration occurred at seventeen weeks, dropping to 714 mg per 100g of tissue. The increase in concentration which follows at eighteen weeks is slight compared to the eight and twelfth week analysis, but the RNA concentration has increased to 753 mg. The next two readings exhibited decreases, 718 and 678 mg for the nineteen and twenty-week-old organisms, respectively. The last organism analysed, twenty-one weeks of age, indicated a definite decrease in RNA content, 798 mg per 100g of tissue.

The average concentration of RNA found in all of the mice analysed for data, both sexes and ranging in age from

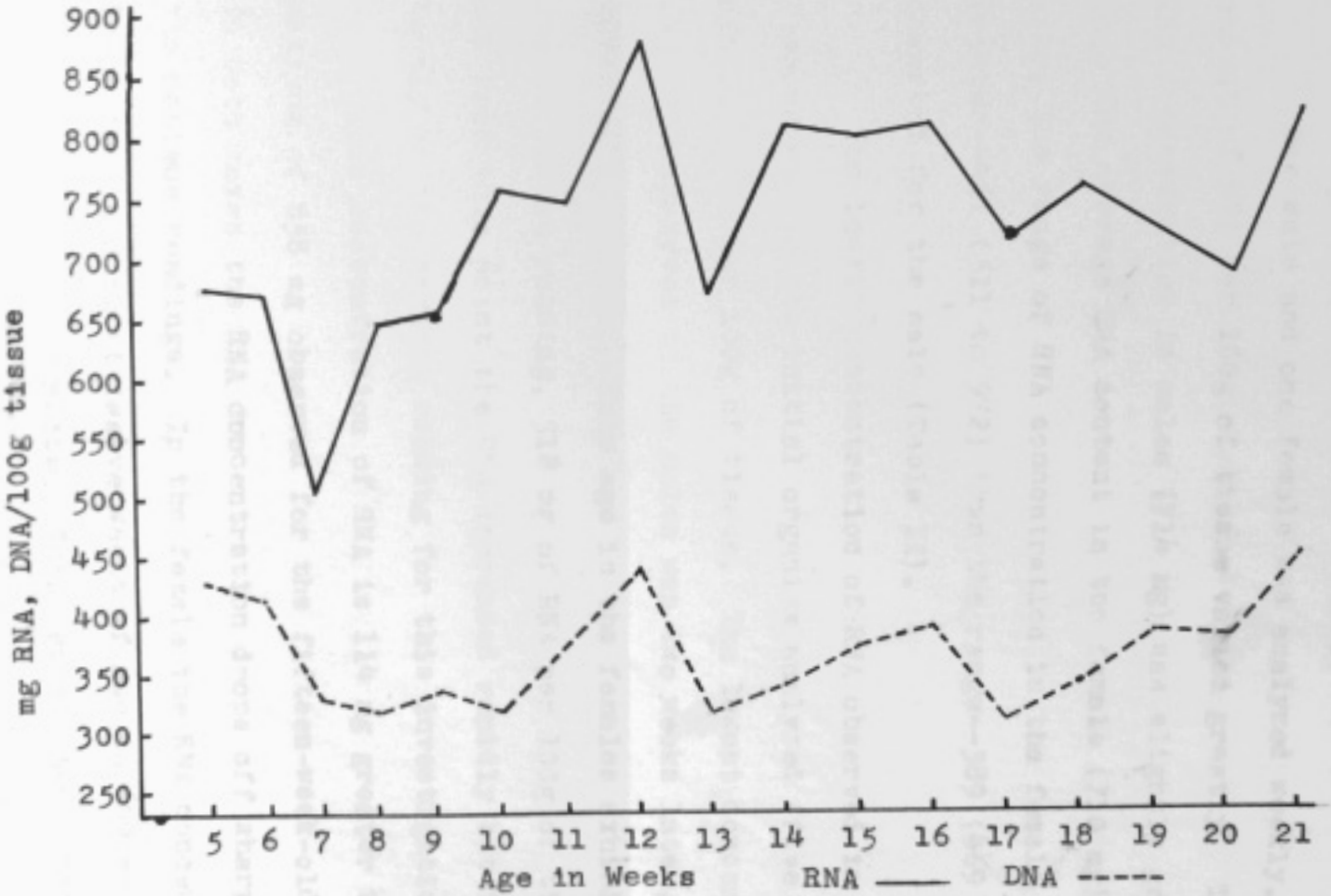

Figure 2. RNA, DNA concentration in untreated mice.

five weeks to twenty-one weeks, in the thirty-four organisms was 712 mg of RNA per 100g of tissue.

One male and one female was analyzed weekly. The amount of RNA per 100g of tissue varied greatly. The average RNA concentration in males (714 mg) was slightly greater than the average RNA content in the female (710 mg). However, the range of RNA concentration in the female was greater--461 (511 to 972) than the range--389 (469 to 858) observed for the male (Table II).

The lowest concentration of RNA observed in the females was in the initial organizm analyzed (five weeks of age), 511 mg per 100g of tissue. The lowest concentration (469 mg) observed in the males was two weeks later, at seven weeks of age. This age in the females exhibited the second lowest reading, 518 mg of RNA per 100g of tissue, and from this point the RNA increased rapidly but inconsistently to its maximum reading for this investigation (972 mg). This concentration of RNA is 114 mg greater than the maximum of 858 mg observed for the fifteen-week-old male. In both sexes the RNA concentration drops off sharply after the maximum readings. In the female the RNA concentration drops from 972 mg at twelve weeks of age to 649 mg for the thirteen-week-old organism. This represented a decrease of 323 mg per 100g of tissue. The RNA concentration in males decreased from a maximum of 858 mg at fifteen weeks

TABLE II

AMOUNT OF RIBONUCLEIC ACID IN NORMAL A/JAX MICE LIVER TISSUE

Age in Weeks	Male	Female	Average* Value
5	821	511	666
6	693	622	657
7	469	518	493
8	612	659	635
9	633	662	647
10	739	738	738
11	681	755	738
12	736	972	854
13	671	649	660
14	780	803	791
15	858	724	791
16	786	808	797
17	657	771	714
18	748	759	753
19	746	690	718
20	671	686	678
21	849	748	798
Average	714	710	712

*Values are given in mg RNA per 100g of tissue.

of age to 657 mg at seventeen weeks of age. This is a decrease of 201 mg per 100g of tissue, after an increase to 748 mg at eighteen weeks. The RNA content in the male exhibited a slight drop to 746 mg at nineteen weeks of age, followed by a definite decrease to 671 mg at twenty weeks. The reading at twenty-one weeks of age represents the second highest concentration of RNA in the male (849 mg). The second highest reading of RNA in the female occurred at sixteen weeks of age (808 mg) and from this point each succeeding reading decreased through the twentieth week, where the decrease reversed to a 748 mg at twenty-one weeks of age (Figure III).

The DNA concentration showed less change than the RNA, but when the DNA decreased, there was a comparable decrease in RNA concentration. Both nucleic acids increased at about the same time (Figure II).

The DNA content for the five-week-old organism was the third highest reading obtained in this investigation--410 mg per 100g of tissue. The succeeding three readings decreased to a low of 305 mg at eight weeks of age. The increase which followed reached its maximum of 425 mg at twelve weeks of age. This increase was not consistant, dropping from 323 mg at nine weeks to 313 mg at ten weeks of age. The thirteen-week-old organism exhibited one of the lower concentrations (318 mg) of DNA, and from this

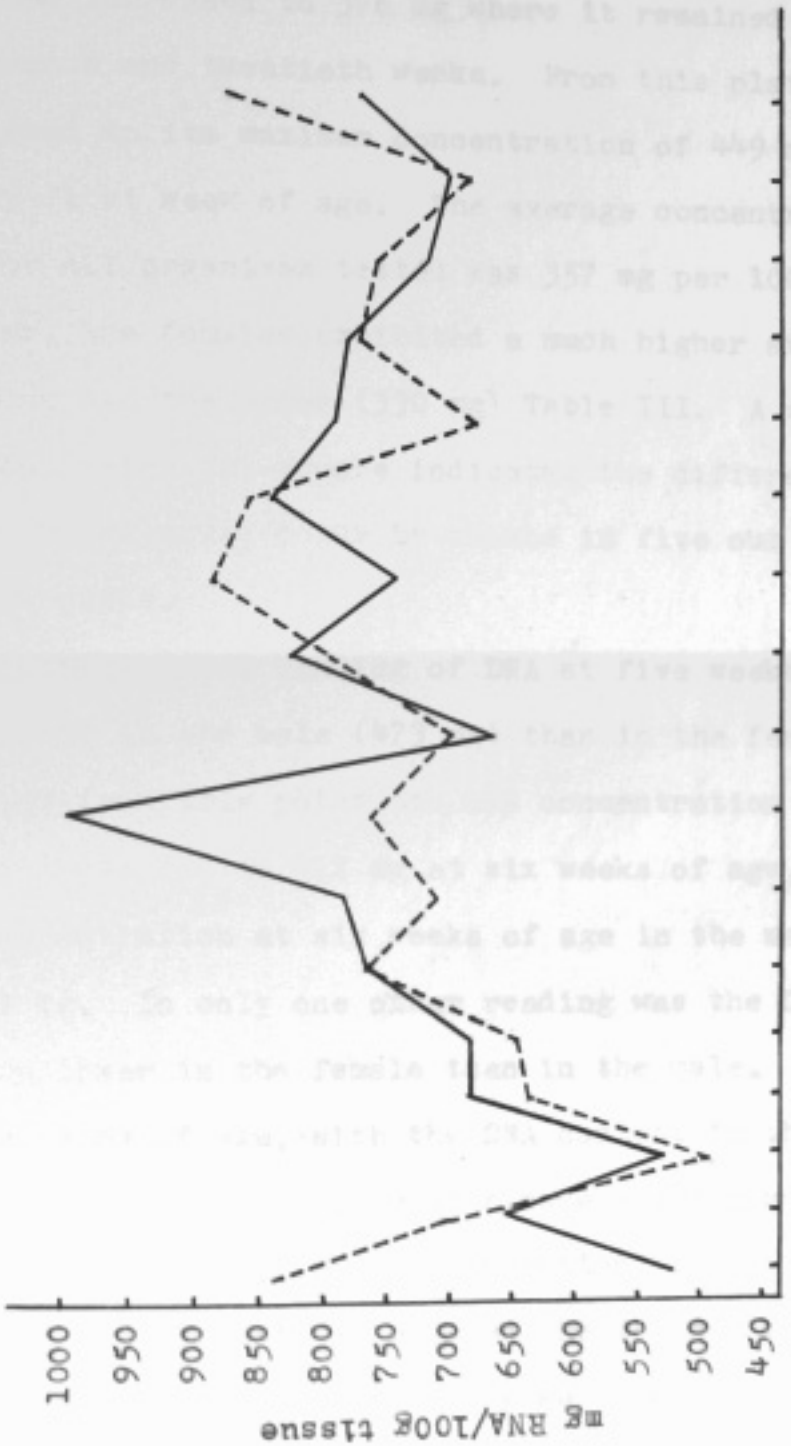

Figure 3. RNA concentration in male and female mice.

point a gradual increase occurred through the sixteenth week, where it reached a peak of 373 mg before it decreased to 299 mg at seventeen weeks of age. The concentration of DNA then increased to 376 mg where it remained for the nineteenth and twentieth weeks. From this plateau the DNA increased to its maximum concentration of 449 mg at the twenty-first week of age. The average concentration of DNA for all organisms tested was 357 mg per 100g of tissue. However, the females exhibited a much higher average (384 mg) than did the males (330 mg) Table III. A statistical analysis using Chi-square indicates the difference between the averages would occur by chance in five out of one hundred cases.

The initial reading of DNA at five weeks of age, was higher in the male (473 mg) than in the female (348 mg), but from this point the DNA concentration in the female increased to 412 mg at six weeks of age, while the DNA concentration at six weeks of age in the male decreased to 371 mg. In only one other reading was the DNA concentration lower in the female than in the male. This occurred at ten weeks of age, with the DNA content in the female exhibiting 305 mg per 100g of tissue and the male 322 mg per 100g of tissue. The concentration of DNA in the female reversed itself until it reached the maximum of 511 mg of DNA per 100g of tissue at twelve weeks of age. The de-

TABLE III

AMOUNT OF DEOXYRIBONUCLEIC ACID IN NORMAL A/JAX MICE LIVER TISSUE

Age in Weeks	Male	Female	Average* Value
5	473	348	410
6	371	412	391
7	310	331	320
8	299	312	305
9	315	332	323
10	322	305	313
11	337	378	357
12	340	511	425
13	291	345	318
14	309	367	328
15	340	373	356
16	332	414	373
17	228	370	299
18	312	351	331
19	305	448	376
20	296	457	376
21	429	469	449
Average	330	384	357

*Values are given in mg DNA per 100g of tissue.

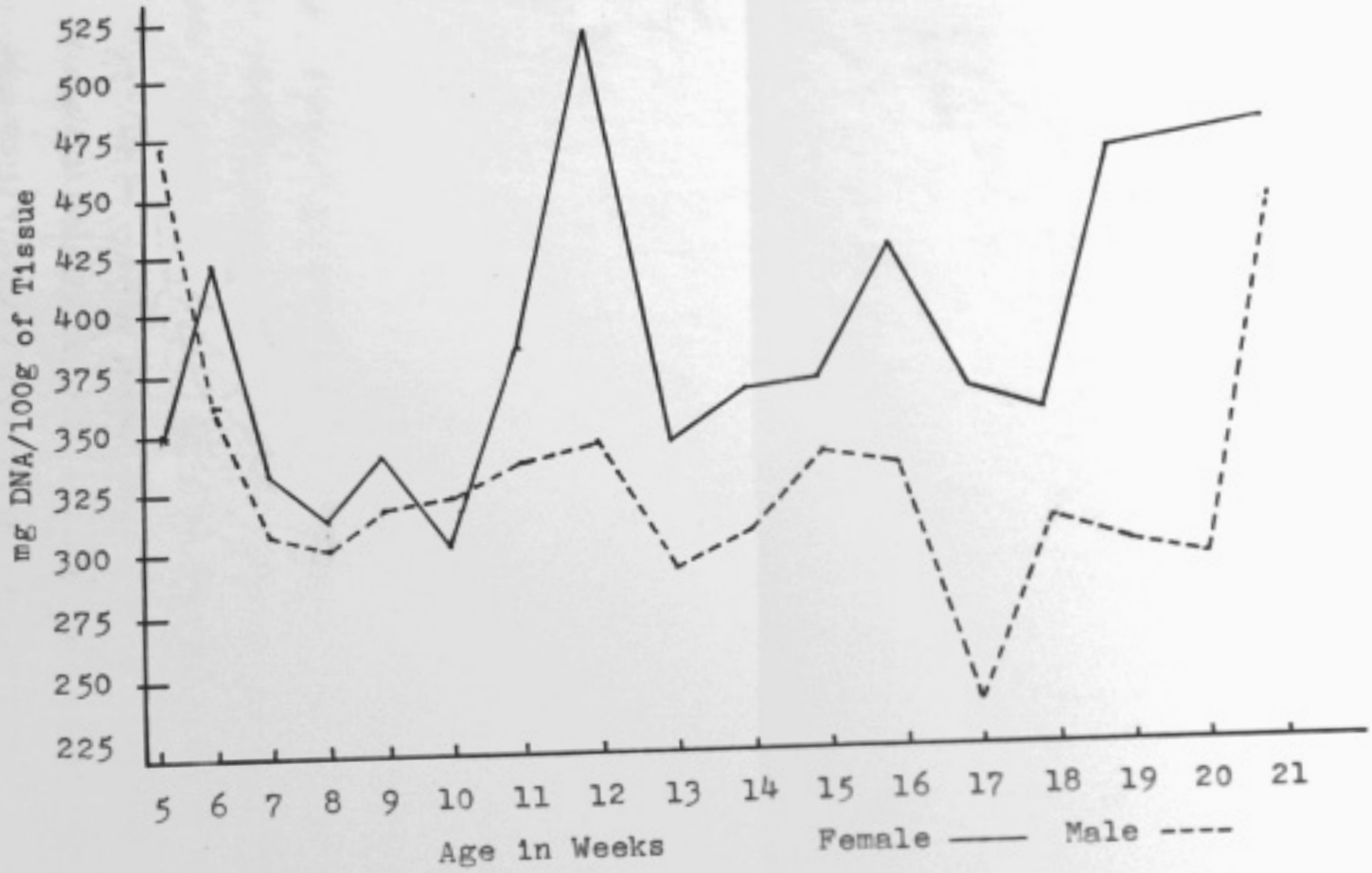

Figure 4. DNA concentration in male and female mice.

crease which occurred at thirteen weeks in the female was an abrupt drop to 345 mg and the recovery from this decrease continued until it reached a concentration of 414 mg at sixteen weeks of age. The concentration of DNA fell to one of the lower readings (351 mg) at eighteen weeks, and the increase which followed reached the second highest reading observed in the females, at the twenty-first week, 449 mg per 100g of tissue (Figure IV).

CHAPTER V

DISCUSSION

Since the metabolism and reproduction of cells involves the interplay between acid soluble phosphates, phospholipids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), a quantitative study of these biochemical constituents was made at Drake University to achieve a better understanding of the changes that occur as cirrhosis and hepatomas develop. Generally, after the twenty-second feeding of carbon tetrachloride, tumors become visible in the livers of A/Jax mice.

Biochemical studies were then made on the mice livers after various numbers of feedings. Determination of the concentrations of the DNA and RNA are shown in Figure I, Chapter II. The fluctuation in the concentration of RNA and DNA which is evident in this report was thought to be caused by the carbon tetrachloride feedings to which the organisms were subjected.

Other studies involving the effects of carbon tetrachloride on liver tissue are in agreement with those performed previously at Drake. One investigation working with rats instead of mice, reported on the cytoplasmic

fractions of the cell. The cytoplasmic RNA, which the investigators report "shows continuous variations from one fraction to another."[1] This investigation was based on cytoplasmic fractions of nine rat livers, while the work at Drake involved the livers of over one hundred mice. However, both investigations exhibited wide variations in RNA content.

In a report on the effects of carbon tetrachloride on the metabolism, Bornig and others reported no significant change in the number of nuclei or the DNA content of the cell.[2] The analysis in this report was made two hours after a single injection of carbon tetrachloride. They indicate, however that there is a slight increase in the DNA content.

Laquerriere states that "after excision of about forty per cent of the liver tissue of white mice there was an increased amount of DNA in the remaining liver tissue about three days after the operation. The synthesis occurred in the cell nuclei and was a prelude to the initiation of the prophase of mitosis. After this initial increase the DNA content reached its maximum a few days later."[3]

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[1] Lamirande, op. cit., pp. 291-292.

[2] Bornig, op. cit., pp. 213-230.

[3] Laquerriere, op. cit., pp. 139-141.

The quoted report lends support to the fluctuations in nucleic acid concentrations observed in previous work at Drake with mice treated with carbon tetrachloride and to the present report on untreated mice.

The fluctuations observed in the RNA, DNA content in the treated mice (Figure I, Chapter II), were reproduced in the same rythmical pattern in the untreated mice. The fact that this rythmical variation occurs over an extended period of time (seventeen weeks in the present study), indicates data collected, concerning nucleic acid concentrations, from a study of shorter duration would give great difficulty in deriving valid conclusions.

The results of the present investigation indicate that the nucleic acids vary in the same rythmical variation in untreated and treated (carbon tetrachloride) mice.

If a direct relationship exists between mitotic cellular division and the amount of nucleic acids present in the cell, it is reasonable to assume the fluctuations observed in previous investigations would be valid and suggest that further study of the relationship that exists between the nucleic acid concentration and the phases of cellular division is warranted.

It has long been established that cellular division occurs at an uneven rate, and that the rate of cellular division is directly related to the physical condition of

the organisms, the rate of metabolism, the age, and sex, as well as to the chemical substances (known and unknown) which are taken in, or manufactured by the body; these would include enzymes, hormones, and vitamins.

In the present study an average DNA concentration of 357 mg per 100g of tissue was observed for the thirty-four mice analyzed in the present investigation. The 330 mg of DNA calculated as the average for the male mice is 54 mg per 100g of tissue less than the average calculated for the female mice (384 mg per 100g of tissue). The difference in the average concentrations of DNA in the male and female mice is sufficient to warrant statistical treatment.

Applying the statistical test (chi-square) it was found that the possibility of the concentration of the DNA in the female being 54 mg per 100g of tissue greater than in the male would occur by chance in less than five out of one hundred instances.

The cyclic pattern observed in DNA concentration (Figure IV, Chapter IV), is not unlike the concentration of leucocytes, cornified and nucleated epithelial cells observed in mice vagina during the normal estrous cycle. The estrogens released during this cycle have long been associated with body changes in the female. These changes have not been pronounced until the concentration of the

hormones have reached a definite level. The fact that these hormones are released in a cyclic pattern may have some effect on the concentration of DNA observed in the present investigation.

It would appear that there is sufficient information to indicate DNA varies to a measurable degree from one organism to another, or within one organism from one time to another.[1]

This information tends to question the hypothesis that the variations observed in the mice treated with carbon tetrachloride were in fact due to the effect of this toxin.

The RNA content has been shown to vary even more than DNA, but apparently no such definite statements have been made (as were made in regard to DNA) to explain this variation.[2] However, it has been generally accepted that RNA follows the pattern established by the DNA; therefore, perhaps the fluctuation of the RNA is an exaggerated form of the same variations observed in the DNA.

The information obtained in this investigation tends to give support to this hypothesis. It should be noted that the figures and tables showing the relationships

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[1] Laquerriere. op. cit., pp. 139-141.

[2] Lamirande, op. cit., pp. 291-292.

between RNA and DNA indicate that the low and high points of the nucleic acid concentrations tend to coincide.

It is apparent that the concentrations of the nucleic acids vary in a rythmical pattern. However, the question still exists as to the cause of the variations. This report has indicated several factors which may affect this concentration. These would be areas where more research is needed. Until it is possible to determine the relationship between hormones, mitoses, normal tissue repair, tumor formation, sex, and the nucleic acids, it appears as though the variations observed in this and previous investigations will not be completely understood.

CHAPTER VI

SUMMARY

The present investigation was initiated in an attempt to establish a relationship in nucleic acid content, RNA and DNA, in the liver tissue of A/Jax mice of both sexes and of various ages. Analyzes were made on mice five weeks through twenty-one weeks of age.

One male and one female was sacrificed each week and analyzed by using a modification of Schneider's[1] procedure for the extraction of nucleic acids.

Information obtained from this investigation exhibits rythmic fluctuations in RNA and DNA concentrations. The RNA appeared to show a greater variance than the DNA, with an average concentration of 714 mg per 100g of tissue in the male and 710 mg per 100g of tissue in the female. The average DNA concentration in the female was 384 mg, which was 54 mg higher than in the male (330 mg). Using the chi-square method of statistical analysis this difference was found to be significant to the extent that it would occur by change in only five out of a hundred cases.

The fluctuation observed in RNA and DNA concentration in the liver tissue of untreated mice parallels the fluctuations indicated in previous studies, especially studies

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[1] Schneider, *op. cit.*, pp. 293-302.

involving treatment with carbon tetrachloride.

A review of the literature indicates that DNA is the cellular substance that establishes the pattern for protein synthesis and that RNA conveys this pattern to the amino acids in the cytoplasm. Over ninety per cent of the RNA is found in the cytoplasm.

Like other investigation, the present investigation indicated a greater variance in RNA concentration than in DNA concentration. Further study of the relationship that exists between these cellular components is clearly indicated. Other areas which require more investigation are the effects of hormones on the nucleic acid concentration with special attention to the effect these substances have on the DNA concentration. This would be especially true of the hormones involved in the estrogen cycle in the female.

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B. UNPUBLISHED MATERIAL

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